Water Quality Planning Bureau

Sample Collection and Laboratory Analysis of Chlorophyll-*a*

Standard Operation Procedure

Approvals: [All Signatures on File at DEQ]

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Chlorophyll-a

Chlorophyll-*a* is measured as a means of estimating algae (periphyton or phytoplankton) biomass in a body of water. It is expressed as either mass/area for periphyton (mg/m^2), or as mass/volume for plankton species (µg/L). Heavy growths of algae generally indicate inferior water quality.

Excess algae growth may clog water filters and irrigation equipment, cause taste and odor problems in water supplies, reduce dissolved oxygen levels, interfere with fish spawning, degrade macroinvertebrate habitat, trap sediment, deflect stream flows, and impair the overall aesthetics of a stream.

1. Scope and Applicability

This method is developed for use in water quality assessments[1] decision-making.

The sampling methods described herein are for *wadeable* streams and rivers. The phytoplankton sampling procedure may be used in low-flow conditions (disconnected series of pools) or in lakes and reservoirs.

These sampling methods are designed to produce a quantitative measure of algae growth by relating the total mass of chlorophyll-a pigment to a known area or volume.

2. Sampling Design Considerations

2.1. Index period

Periphyton growth is controlled by season, nutrient concentrations, velocity of the current, days of accrual, grazing, shading, water temperature, and other factors. Because of this, sampling designs using chlorophyll-a must be inclusive of the times when stable flows have been achieved, as well as times when diversity and standing crop are peaking. Intensive sampling may include multiple visits to show the water body's baseline condition, period of high growth potential, and subsequent return to baseline conditions. The summer period of June 21 to September 21 is generally the time of maximum growth potential in western Montana (mountainous region). A recent study (Suplee 2004) performed by DEQ in the northern glaciated plains used an index period of May – September to represent the period of maximum growth.

2.2. Recent conditions

Sampling events planned in advance must consider the possibility that current or recent weather patterns could influence the outcome. An example of this is recent or current rainfall that significantly increases the flow, scouring the substrate. If the water body has had recent significant rainfall or is currently experiencing a significant rainfall event, consider the effect of scouring and reschedule sampling event, if necessary.

2.3. Site Locations

Selection of sampling *locations* depends largely on the data quality objectives (DQOs) of the water quality study. The study design necessary to satisfy these DQOs must be documented in a project plan (QAPP, SAP, or equivalent documentation). The project plan should have sufficient detail to allow minor adjustments (to pre-selected sites) in the field due to unforeseen events such as site inaccessibility.

[1]SOP WQPBWQM-001 – Montana Department of Environmental Quality, Water Quality Assessment Process and Methods.

If sampling locations are to be determined in the field, field guidance should include rationale for site location. This is critical when different sampling crews select representative locations based on professional judgment.

2.4. Geo-locating site

The first measurement collected is a geo-reference for the study site.

Once the site is located in the field, this location must be taken using a GPS as per DEQ SOP OIT-001 (available online at http://10.194.19.212/gis/gps/GPSSOP_1_06.pdf)

The location recorded for a chlorophyll-a sampling event is the #F (middle) transect (layout of the sampling unit is discussed in section 2.5). This is consistent with the approach used in EPA EMAP (Lazorchak, Klemm, and Peck 1998) studies as well as DEQ's Reference Project. The #F location will be used for geo-referencing the site for EPA's STORET database.

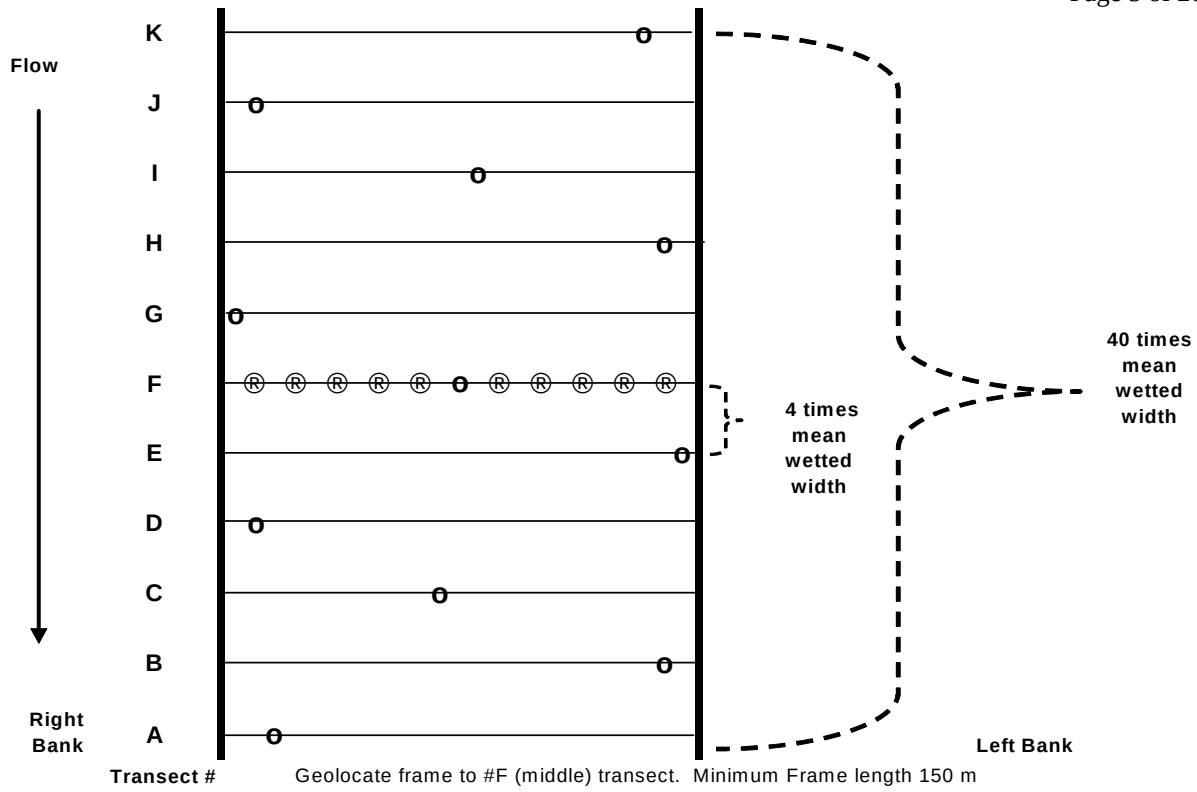
2.5. Sampling Unit

Beginning with the 2006 field season, an 11 transect sampling unit will be used at each site. This unit consists of 11 transects with 40 wetted widths defining the total unit (Figure 1.0). Small streams must use 40 wetted widths or a minimum of 150 meters whichever is larger. Since several rivers are wide and shallow, 40 wetted widths may entail several kilometers, which may be unmanageable. Therefore, if the sampling unit would exceed 500 meters then it is acceptable to switch to the large river method. This allows taking transect samples across the river at a defined point (#F in Figure 1.0).

The procedure for determining the distance between transects is to measure the wetted width at 5 places from the F site (2 upstream, 2 downstream, and 1 at the F site), average the five readings and round to the nearest 1 m.

Samples taken at each location within the unit are *single* collections using the appropriate collection technique for the substrate encountered (listed in Section 3 - Sample Collection Methods). The starting point right (R), center (C), left (L) should be randomly selected for the most downstream transect (#A). Place sampling locations progressing upstream following the R, C, L pattern.

Figure 1.0 - Diagram of chlorophyll-a sampling unit (wadeable streams & rivers)



o - designates sample locations, wadeable streams and rivers
® - designates sample locations, large wadeable rivers

Note: If the sampling unit exceeds 500 m switch to large river method (eleven evenly spaced samples taken *across* the river/stream at site #F.)

2.6. Sampling Quality Control

The appropriate Quality Control samples to assess field collection activities must be designated in the project planning documents (QAPP, SAP). Because the designated sampling unit is a multi–transect sampling, information about the variability among measurements is inherent to the collection design, therefore, duplicate samples do not *generally* need to be collected unless project DQO's require a high degree of defensibility. Documentation of the approach planned to evaluate the results should be described in the quality control section of the project planning documents.

2.7. Data Review and Evaluation

Standards are based upon the arithmetic mean of the eleven individual results. If samples are composited according to type (core, hoop, template), a weighted average (Section 6.2.4) is obtained based on the number of transects per collection method to determine the chlorophyll-a concentration.

3. Collection Methods

Periphyton standing crop may be quantified by measuring the amount of accrual on natural substrates at the study site. The sampling of artificial substrates is not recommended.

There are three methods for collecting attached algae (periphyton) – hoop, core, and template from streams and rivers. A single sampling using one of the listed collection techniques is performed at a transect. The substrate and conditions encountered at the sampling point on the transect determines the collection technique.

Sample compositing may be used to reduce the costs associated with the 11 samples collected as part of the sampling unit described in Section 2.5. Sample compositing will, in effect, return results of each collection method as *mean* when the composite chlorophyll-a concentration is calculated to the sum of the areas collected. **ONLY SAMPLES COLLECTED BY THE SAME TECHNIQUE CAN BE COMPOSITED!** There are three sampling techniques used in this method (Hoop, Core, and Template). Therefore, there may be up to *three* composites resulting from the 11 transect sampling.

The 11 transect sampling unit (section 2.5) is required for all collections. It has been demonstrated that this design will generally encounter one to three extreme values. Analyzing each of the 11 samples separately allows the assessor to understand the patchiness of algal growth. However, the data should be considered using a reach average (Mean).

If the water is too deep (greater than 2-3 feet) in the location you are sampling (R, C, L), adjust the sample location in the direction of the pattern until depth is acceptable for sampling.

3.1. Template sampling method

The template method is used for sampling transects with substrate dominated by small boulders, cobble and gravel *without heavy filamentous growth*.

3.1.1. Method Summary

A template with a 12.5 cm^2 area is placed on a rock with representative algae density at the designated point on the transect line. The sampler should observe the algae density in a roughly 1 meter by 1 meter area centered on the sampling point and select a representative rock therein. The area within the template is scraped into a container and filtered on site (0.70 um GFF). At the laboratory, the filter will be extracted and the resulting extract measured for chlorophyll a. The template can be made, for example, of a cut-off piece of PVC pipe (Schedule 40 - 1 1/2" nominal I.D.), which results in an area of approximately 12.5 cm^2. Internal diameter of template should be checked and be within 3.93 to 4.05 cm (+/- 4% area error).

3.1.2. Sampling Equipment

- Waders or hip boots
- 50 cc centrifuge tube
- Knife for scraping rock
- Tooth brush for brushing rock
- Tap water in squirt bottle
- Shallow plastic pan to hold rock
- Hand pump vacuum with tubing
- Nalgene filtering unit
- GFF filters (0.70 um)
- Tweezers or forceps

- Cooler with ice or dry ice (preferred)
- Aluminum Foil
- Large and medium Ziploc bags
- Sharpie

3.1.3. Sample Collection

A representative rock is placed in the shallow pan and the template placed over the upper (light-facing) surface of the rock. All of the growing material within the template is scraped and placed in the pan.

In certain cases the volume of algal material on the rock surface is small, therefore it is better to scrub the rock surface with a toothbrush and then rinse the rock surface and the toothbrush into the pan with a small volume of **tap** water (Note: Previous versions of this SOP listed de-ionized water. DO NOT USE de-ionized water as it may burst cells due to osmotic pressure differences.)

In some cases, the rocks are very small (smaller than template diameter but too large for core sampling), in this case, instead of using 1 representative rock, place several small representative rocks inside the template diameter, and follow the process as described in the above paragraphs.

Field filtration (MUST BE PERFORMED IN THE FIELD)

The rinse water/algae material that has been rinsed into the pan may be field filtered onto a GF/F filter and the filter placed in the centrifuge tube. Refer to Section 3.4.3 for proper use of the Nalgene filtering unit.

3.1.4. Sample Handling & Labeling

Place all the rinsed material (or GF/F filter) into an appropriate size container (centrifuge tube for GF/F filers). Sampling location is identified on an external label with the following information:

a) Sample Type
b) Activity ID
c) Collection Date
d) Waterbody Name
e) Collector's Name

Fill out the outside label, place it on the centrifuge tube or container, and cover the label completely with a strip of clear tape. Wrap the tube with aluminum foil to exclude light, write the Activity ID on the lid with a **SHARPIE.** Place the centrifuge tube or container into a Ziplock plastic bag.

Immediately store the sample on dry ice or ice and away from light. Samples should be sent to the laboratory as soon as possible for chlorophyll-*a* analysis.

Record the transect number (A-K), collection position (Right, Center, Left), the collection technique (C = Core, H = Hoop, T = Template) in the SVF/COC. You must record this information even if your samples are composited (Section 4.2.1). If the corresponding surface area is different than the one indicated in this SOP, you must record it on the SVF/COC.

3.2. Hoop Method

The hoop method is designed for transects *dominated by the presence of filamentous algae*, regardless of stream substrate.

3.2.1. Method Summary

The hoop collection method is a sample from a representative area where filamentous algae dominates the sampling point, regardless of stream substrate. Upon collection, filamentous algae is physically separated from macrophytes (retain both portions if Ash Free Dry Weight is being analyzed). The filamentous algae sample is submitted to the laboratory for extraction and analysis of chlorophyll-a. The macrophytes portion may be analyzed by ash free dry weight to determine macrophyte biomass.

3.2.2. Sampling Equipment

- Waders or hip boots
- Large freezer storage bags
- Aluminum Foil
- Cooler with ice or dry ice (preferred)
- Metal hoop (30 cm diameter, 710 cm^2 area)
- Scissors
- Tooth brush
- Knife for scraping rocks
- Sharpie

3.2.3. Sample Collection

Find designated location in the transect line. Within an area of +/- 1 m^2 locate a representative area to place the hoop. If a small number of macrophytes (< 5% by area) are present, they can be separated from the filamentous algae sample at the time of collection. If >5% macrophytes are present, collect the sample and perform the filamentous algae separation on the bank using a pan or bucket placed on a stable surface.

Place a metal hoop[2] over the representative area. All the algal material within the hoop is collected (i.e. filamentous and non-filamentous). Scissors or a knife may be used to detach the filamentous algae from their substrate. Algae attached to rocks within the hoop are scraped into the Ziplock bag. Minimize the amount of water submitted by decantation (do not decant floating algae)

3.2.4. Sample Handling & Labeling

Place all filamentous algae collected at the site into a large Ziploc freezer bag. Sampling location is identified on an external label with the following information:

a) Sample Type
b) Activity ID
c) Collection Date
d) Waterbody Name
e) Collector's Name

[2] The hoop can be made by wrapping a stiff wire around the bottom of a 5 gallon bucket. Check area by measuring hoop diameter and calculating for area of a circle ($A=3.14*(D/2)^2$), adjust as necessary to arrive at an area of 710 cm^2. The diameter of the hoop is approximately 30 cm.

Fill out the outside label, place it on Ziploc bag, and cover the label completely with a strip of clear tape. Wrap the bag with aluminum foil leaving no space for light to enter. Place this wrapped bag into another large Ziploc bag and hand write the Activity ID on the outer bag with a **SHARPIE.** Immediately store the sample on dry ice or ice and away from light. Send samples to the laboratory as soon as possible for chlorophyll-*a* analysis.

Record the transect letter (A-K), collection position (Right, Center, Left), the collection technique (C = Core, H = Hoop, T = Template) in the SVF/COC. You must record this information even if your samples are composited (Section 4.2.1). If the corresponding surface area is different than the one indicated in this SOP, you must record it on the SVF/COC.

3.3. Core Method

Method for transects dominated by silt-clay substrate without heavy filamentous growth.

3.3.1. Method Summary

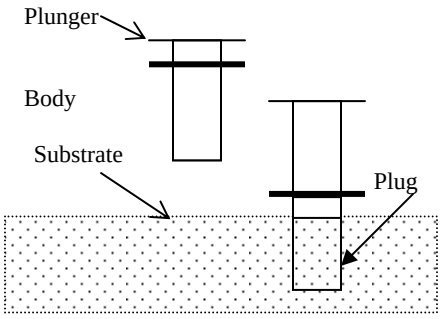
The core collection method is a sample from a representative area where a silt-clay substrate dominates the sampling point on the transect, and luxuriant plant growth is not present. A core sample is taken from the substrate. The top 1cm of the core is sliced off the plug and placed in a centrifuge tube. The sample is sent to the laboratory for extraction & analysis.

3.3.2. Sampling Equipment

- Waders or hip boots
- Cut-off 60 ml syringes (5.6cm^2)
- 50 ml centrifuge tubes
- Aluminum foil
- Small Ziploc bags
- Knife
- Sharpie

3.3.3. Sample Collection

A 5.6 cm^2 core sample is collected using a cut-off 60 cc syringe in a representative portion of the designated transect site location.



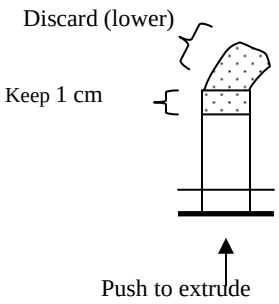
Each core sample is taken by driving the 60 cc syringe into the substrate to a depth of 5-10 cm. The syringe plunger may have to be drawn up as the body of the syringe sinks into the substrate to accommodate the core sample "plug". (The plunger may have too much friction within the barrel to rise on its own as the body of the syringe is pushed into the sediment.)

The plug may be comprised of loose sediment that will fall out of the syringe. To minimize loss of a loose plug, the sampler may have to place a finger over the end of the syringe as it is pulled out of the hole and up through the water column.

In deeper water, a broom handle can be duck-taped to the syringe to extend your reach.

Immediately invert the syringe containing the plug to prevent the plug from sliding out of the barrel.

Extrude the core so the upper 1 cm of the core remains in the syringe. Slice off and discard the lower portion. Place the 1 cm portion in a 60 ml centrifuge tube.



Important: Only the **upper 1 cm** of each core sample is placed in a centrifuge tube. Assure that all the material adhering to the surface of the plunger-end is carefully collected, as most of the chlorophyll is attached.

3.3.4. Sample Handling & Labeling

Sampling location is identified on an external label with the following information:

a) Sample Type
b) Activity ID
c) Collection Date
d) Waterbody Name
e) Collector's Name

Fill out the outside label, place it on the centrifuge tube, and cover the label completely with a strip of clear tape. Wrap it with aluminum foil to exclude light, write the Activity ID on the lid with a **SHARPIE.** Place the centrifuge tube or container into a self-sealing plastic bag.

Immediately store the sample on ice and away from light. Samples should be sent to the laboratory as soon as possible for chlorophyll-*a* analysis.

Record the transect letter (A-K), collection position (Right, Center, Left), the collection technique (C = Core, H = Hoop, T = Template) in the SVF/COC. You must record this information even if your samples are composited (Section 4.2.1). If the corresponding surface area is different than the one indicated in this SOP, you must record it on the SVF/COC.

3.4. Phytoplankton Method (chlorophyll-a in water)

The phytoplankton method is the sampling method for determining chlorophyll-a in the water column. It is used for transects dominated by pools with green color (light green to dark green) and lakes.

3.4.1. Method Summary

This method uses a filtration apparatus to collect a sample. Since chlorophyll-a breaks down readily in sunlight, the use of a **dark Nalgene bottle is required** to minimize the exposure of the sample to sunlight. The filter apparatus should be set up prior to sample collection to minimize time between sampling and filtration. The volume of water filtered must be recorded!

3.4.2. Sampling Equipment

- 50 ml centrifuge tubes
- 1 - hand pump vacuum with tubing
- Nalgene filtering unit
- GFF filters (0.70 um)
- Tweezers or forceps
- Graduated cylinder (100-250 ml)
- Tap water in squeeze bottle

- 1 L (Dark) Nalgene Bottle
- Sharpie

3.4.3. Sample Collection

Filter apparatus setup

Using clean forceps, place a glass fiber filter (GF/F nominal pore size 0.7 um) on the filter holder. Use a small amount of tap water from a wash bottle to settle the filter.

Rinse the sides of the filter funnel and filter with a small volume of tap water.

Sample collection and processing

Rinse a 1L dark Nalgene bottle 3 times with stream or lake water before collecting the sample.

Grab a water sample from an undisturbed location using the 1L Nalgene bottle. Cap the bottle and invert the bottle 3 times to mix thoroughly.

Rinse a 100-250 ml. graduated cylinder three times with *tap water*.

Measure 20 ml or more of sample in the graduated cylinder and pour into the filter funnel, place the cap on the filter funnel. Draw the sample through the filter using the vacuum hand pump. **Note: To avoid rupture of fragile algal cells, DO NOT EXCEED 9 inches Hg on the pressure gauge.**

Keep track of the volume of sample filtered! The volume of sample filtered may vary from 5 ml to 1000 ml or more. When filtration slows and the filter has developed a distinct green (or green-brown) color, sufficient sample has been filtered. Do not allow the filter to clog. If a filter completely clogs while water remains in the upper half of the apparatus, discard the filter and start again, use less water volume.

After filtration is complete, unplug the hand pump, remove the filter funnel from the filter holder, and remove the filter with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored side folded on itself. Place the folded filter paper in a 50 ml centrifuge tube.

3.4.4. Sample Handling & Labeling

Sampling location is identified on an external label with the following information:

a) Sample Type
b) Activity ID
c) Collection Date
d) Waterbody Name
e) Collector's Name

Fill out the outside label, place it on the centrifuge tube, and cover the label completely with a strip of clear tape. Wrap it with aluminum foil to exclude light, write the Activity ID on the lid with a **SHARPIE.** Place the centrifuge tube or container into a self-sealing plastic bag.

Immediately store the sample on dry ice or ice and away from light. Samples should be sent to the laboratory as soon as possible for chlorophyll-*a* analysis.

4. Recording the chlorophyll-a sampling event

The chlorophyll-a sampling event must be recorded including information on the location, collection methods used, and the area/volume collected. DEQ uses a Site Visit Form (SVF - Attachment 1) to record this information in the field for later entry into EPA's STORET database.

4.1. Geo location

As described in Section 2.4 of this SOP, the #F transect is used to geo-reference the sample site. Other locations may be recorded to document the extent of the sampling unit, however, these must be clearly distinguished so that the #F middle transect is easily recognized. Regardless of how many locations are recorded, in STORET, only the #F middle transect will be used to geo locate the site.

4.2. Recording the sampling event - DEQ Site Visit Form (SVF)/Chain-of-Custody

DEQ established a site visit form for recording monitoring sampling event metadata. This form was designed to geo-locate a sampling event to the single, downstream site in multi-parameter sampling events. Therefore, when using the DEQ site visit form, it is imperative that the geo-location of the #F site for a chlorophyll-a sampling event is distinguished from the latitude and longitude of the other measurements. A Site Visit Form is provided as Attachment 1 to this SOP.

4.2.1. Site Visit Form

Indicate a chlorophyll-a sample was taken by checking the chlorophyll-a box. Note the *Sample ID* on the Site Visit Form. Indicate the sample collection procedure and location used at each transect on the Site Visit Form using the following abbreviations. The first letter represents the sample type and the second letter represents the location on the transect.

First letter (Technique/Type)

- Template = T
- Hoop = H
- Core = C

Second letter (Position)

- Right = R
- Left = L
- Center = C

For example: Transect: A_C-R_, B_H-C__, C_C-L__, D_T-R__, Etc,

Record the geo-location of the #F site under Site Visit Comments. Record the mean wetted width from the #F site (Section 2.5 Sampling Unit) under Site Visit Comments.

4.2.2. Site Visit Forms (SVF)/ Chain of Custody

Include each sample on a separate line and include the area or volume collected so the laboratory may complete the calculations to area or volume. Phytoplankton samples must include documentation of volume filtered. ***The laboratory must not accept samples until the field crew provides these documents.***

4.2.3. Compositing

If compositing is desired, THIS FACT MUST BE NOTED ON THE SVF/COC. Samples for composite will be collected within the 11 transect sampling unit using the appropriate collection

technique for the substrate encountered. Because chlorophyll-a readily breaks down in sunlight, samples must be composited in subdued light at the laboratory prior to processing. Samples collected by different techniques CANNOT be composited. However, method-specific composites may be made (i.e. Core Composite, Hoop Composite, and Template composite).

The sampler is responsible for providing the area, type (core, hoop, template), and the number of each type of each individual sample collected and recording this on the SVF/COC. The laboratory is responsible for multiplying the number of composites by the area of each sample for determining the denominator of the final result.

5. Sample Extraction

Sample extraction and spectrophotometric determination are to be performed in an analytical laboratory by a qualified laboratory technician or chemist. Sample extraction and determinative techniques described herein are *modified* from the procedure described in EPA 446.0 (Arar E. 1997). These modifications are:

- Use of the monochromatic equation for phaeopigment-corrected chlorophyll-a with the extraction solvent *ethanol* (Suplee, Watson, and et *al* 2006), and
- Option for using HPLC.

The solvent purity and grade used for extraction can greatly influence the outcome of the analysis. Therefore, this procedure limits extraction solvent options to those listed in Table 1 (page 17). If HPLC will be used, strict adherence to solvents and instrument conditions described in Standard Mwethods 10200H (5) must be maintained.

Caution: ALL CHLOROPHYLL WORK MUST BE PERFORMED IN SUBDUED LIGHT

If processing must be delayed, hold solid/filter samples at -20^{o}C and protect them from exposure to light. Solid/filter samples taken from water having a pH 7 or higher may be placed in airtight plastic freezer bags and stored frozen for 3 weeks. Samples from acidic water must be processed promptly to prevent chlorophyll-a degradation.

The four different sampling techniques result in different types of media being submitted to the laboratory, and extractions to accommodate each media follow.

5.1. Hoop Samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. A hoop sample consists of all filamentous algae collected in a 710 cm^2 area. Samples are shipped to the laboratory in a ziploc bag covered with aluminum foil, and with a second (outer) ziploc bag protecting the aluminum foil. Upon arrival at the laboratory, samples may be analyzed immediately or frozen until ready for analysis. Frozen samples must be thawed in subdued light at room temperature.

5.1.1. Sample Extraction

- If frozen, remove sample from freezer and allow it to thaw in subdued light at room temperature.
- Remove any excess water by straining or decanting. Excessive water interferes with the spectrophotometric measurement.

- Add enough solvent to the (inner) ziplock bag to cover the sample. Record volume added. A minimum of 13 ml of solvent is needed for analysis. Additional solvent may be added, however the more dilute the sample becomes, the lower the instrument reading will be relative to the calibration range of the spectrophotometer - *don't dilute into a non-detect at extraction.*
- Samples may require grinding to break cell walls to release chlorophylls
- Identify the sample with a label.
- Keep in the dark overnight.
- The next day, proceed with spectrophotometric analysis (Part 6 of this SOP)

5.2. Core Samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. A core sample is the *upper 1 cm* of a 5.6 cm^2 core plug. The sample will be returned from the field in a 50 ml centrifuge tube wrapped in aluminum foil. This foil wrapped tube should be in a protective (outer) Ziploc bag. Sample should arrive frozen and remain frozen until ready for extraction.

5.2.1. Sample Extraction

- When ready to extract, remove sample from freezer and allow it to thaw.
- Add enough solvent to the centrifuge tube to cover the sample. Record volume added. A minimum of 13 ml of solvent is needed for analysis. Additional solvent may be added, however the more /solvent added, the greater the dilution of pigments – *don't dilute into a non-detect.*
- Core samples can be very thick and compact. It may be necessary to mix the solvent and sample using a mechanical shaker.
- Keep in the dark overnight.
- The next day, proceed with spectrophotometric analysis (Part 6 of this SOP)

5.3. Template Samples

Template samples must arrive at the laboratory as a frozen filter sample. This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment.

Template samples are scrapings and subsequent washing of the rock within the template area. The water and scrapings are then filtered. The filter is submitted to a laboratory who extracts the chlorophyll-a from the filter and analyzes the extract. Field crews must have filtration equipment available and return samples (on filters) in centrifuge tubes. The template area must be included on the SVF/COC prior to lab proceeding with the analysis because results are reported as mass/area. Filtered samples should be returned to the laboratory frozen and remain frozen until analysis.

5.3.1. Sample Extraction

- Assemble filtration apparatus and quantitatively transfer and filter entire sample through a 47 mm glass fiber filter with a nominal pore size of 0.7 um. (Whatman GF/F filters)
- Volume filtered is irrelevant because results will be related to template area.
- Place filter into a labeled centrifuge tube.
- Add a minimum of 13 ml. of solvent to centrifuge tube and keep in the dark overnight. Do not add more than 20 ml of solvent unless multiple filters were required for the sample. Make sure the entire filter is covered by solvent. Record volume of solvent added.
- Keep sample in the dark overnight.

- The next day, proceed with spectrophotometric analysis (Part 6 of this SOP)

5.4. Phytoplankton Samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. Phytoplankton samples may arrive at the laboratory as either a water sample or as a filter in an aluminum foil wrapped centrifuge tube. Filtering of water samples should be performed as soon as samples arrive since algal populations may change in a relatively short period of time. **Filtration in the field is preferred.**

5.4.1. Sample Extraction

Filter apparatus setup (lab filtration of phytoplankton or template samples submitted as aqueous samples)

- Using clean forceps, place a glass fiber filter on the filter holder. Use a small amount of tap water from a wash bottle to settle the filter. (Note: Previous versions of this SOP listed de-ionized water. **DO NOT USE de-ionized water as it may burst cells due to osmotic pressure differences.)**
- Rinse the sides of the filter funnel and the filter with a small volume of tap water.
- Cap the bottle and thoroughly mix by inverting bottle 3 times.
- Rinse a 100-250 ml. graduated cylinder three times with tap water.
  Measure 20 ml or more of sample in the graduated cylinder and pour it into the filter funnel, place the cap on the filter funnel, and draw the sample through the filter using the vacuum hand pump. **Note: to avoid rupture of fragile algal cells, DO NOT EXCEED 9 inches Hg on the pressure gauge.**
- **Keep track of the volume of sample filtered!** The volume of sample filtered varies from 5 ml to 1000 ml or more. When filtration slows and the filter has developed a distinct green (or green-brown) color, sufficient sample has been filtered. Do not allow the filter to clog. DO NOT DISCARD excess sample (water) from the filtration apparatus. A portion of the sample from the filter media will be lost (this also makes it difficult to keep track of volume filtered.). If a filter completely clogs while water remains in the upper half of the apparatus, discard the filter and start again, use less water volume.
- After filtration is complete, unplug the vacuum pump, remove the filter funnel from the filter holder, and remove the filter with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored site folded on itself. Place the folded filter paper in a 50 ml centrifuge tube.
- If filter extract is to be performed immediately, proceed with filter extraction. If filter extraction will be delayed, cover centrifuge tube with aluminum foil and freeze sample for extraction (maximum 3 week storage).

Filter Extraction

- When ready to extract, remove sample from freezer and allow it to thaw.
- Add enough solvent to the centrifuge tube to cover the sample. Record volume added. A minimum of 13 ml of solvent is needed for analysis. Additional solvent may be added, however the more solvent added, the greater the dilution of pigments – *don't dilute into a non-detect.*
- It may be necessary to mix the solvent and sample using a mechanical shaker.
- Keep in the dark overnight.
- The next day, proceed with spectrophotometric analysis (Part 6 of this SOP)

6. Spectrophotometric Determination of Chlorophyll-a

The Spectrophotometric determination of chlorophyll-a is to be performed according to EPA Method 446.0 (Arar E. 1997) or Standard Methods 10200 H (APHA 1998, 20th). Both of these methods include the monochromatic calculation required for spectrophotometric analysis with phaeophytin-a correction.

Each laboratory must have current standard operating procedures (SOPs) that describe their instruments, reagents, interferences, standards, instrument setup, calibration procedures, analytical procedures, quality control requirements, calculations, and reporting protocols. Except as provided below, these SOPs must describe a method in general accordance with the reference methods EPA 446.0 or Standard. Methods 10200H.

A reference sample must be run with each analysis to determine method bias at a 10% frequency. The reference may be purchased from Sigma Aldrich or any certified vendor. Acceptance limits are +/- 20% of True Value. Control charting of reference sample performance is suggested to better control method performance.

Chlorophyll-*a* methods in general list Absorbance Correction Factors (ACF) or give values for k and A. It is preferred that the laboratory calculate the values of k and A from average values obtained by analyzing 20-30 aliquots of a reference material. This will allow the calculation of a method ACF specific to the laboratory's instrument and purity/grade of reagents used. If reference values (Table 1) from literature will be used rather than calculating its own ACF, the laboratory must demonstrate acceptable method performance in an initial method validation and re-establish this acceptable performance annually or as changes in instrument conditions or reagents require. Above all, recognize the potential for high or low bias to exist in this method and do not blindly follow published or literary values for the ACF without verification.

$$\text{Absorbance Correction Factor} = k \times A$$

$$k = \frac{(664b/665a)}{(664b/665a)-1}$$

$$A = \frac{l\,(cm) \times \text{Concentration (mg/l)}}{\text{Absorbance 664b}}$$

6.1. Correction for pheophytin-a

Both reference methods (EPA 446.0 and Standard Methods 10200H) provide calculations for obtaining monochromatic (chlorophyll-a corrected for presence of phaeophytin-a) and trichromatic (chlorophyll-a,b,c) results. Montana law (ARM 17.30.602(4)) requires that chlorophyll-a water quality measurements are corrected for phaeophytin (pheophytin).)

Refer to Standard Methods 10200H(2) (APHA 1998, 20th) or EPA Method 446.0 (Arar E. 1997), Section 12.2 for instrument requirements, sample analysis requirements (calibrations, reagents, wavelengths, and calculations).

The calculations presented in the reference methods are for a phytoplankton (water) sample and can be applied directly for those samples.

Chlorophyll-a mg/m^3 = [(Absorbance Correction)((664b-750b)-(665a-750a))*V1/(V2*L)]

Where: V1 = Volume of extract (L)
V2 = Volume of sample (m^3)
a = after acidification
b = before acidification
L = Light path or width of cuvette , cm

The calculation for periphyton replaces area for volume.

Chlorophyll-a mg/m^2 = [(Absorbance Correction)((664b-750b)-(665a-750a))*V1/(A1*L)]

Where: V1 = Volume of extract (L)
A1 = Sample collection Area (m^2)
a = after acidification
b = before acidification
L = Light path or width of cuvette , cm

The allowed solvents in its purity form are listed in Table 1.

Table 1 Approved Solvents and Absorbance Correction Factors.

SOLVENT PURITY	SOLVENT	ABSORPTION PEAK RATIO(APR)	SPECIFIC ABSORPTION COEFFICIENTS (E_{1CM})	A	K	ABSORBANCE CORRECTION = (A X K)
90%	Acetone (Ace)	1.7 note 1	89.0 L/(g*cm) note 2	11.0 note 1	2.43 note 1	26.7 note 1,4
95%	Ethanol (EtOH)	1.72 note 2	83.4 L/(g*cm) note 2	11.99 note 3	2.39 note 3	28.6 note 3

1. APHA 1998
2. Values listed by Sartory (Sartory D.P. and Grobbelaar J.U. 1984:177-187)
3. Calculated from values listed by Sartory (Sartory D.P. and Grobbelaar J.U. 1984:177-187)
4. Significant figure error. Error carried forward.

6.2. Calculation to area

In order to determine the density of periphyton algae by measuring chlorophyll-a, results obtained from the instrument (in mg) must be related to the area sampled rather than a volume of water. The area obtained from the three collection techniques varies. If the area information is not readily available on the COC, the laboratory must *not* begin the extraction and analysis until it is provided.

Also, if compositing is used, the number of composites and total area sampled must be submitted on the chain-of-custody/svf.

6.2.1. Area of Hoops

A hoop has a standard area of 710 cm^2. *Confirm area of hoop prior to use*. If compositing is used, the number of hoop samples composited must be confirmed from the SVF/COC. Calculate as follows:

$$\frac{710\ cm^2 \times "number of hoops"}{10{,}000\ cm^2 / m^2} = X\ m^2$$

For example, for one hoop: $\frac{710\ cm^2 \times 1}{10{,}000\ cm^2 / m^2} = 0.0710\ m^2$

6.2.2. Area of Cores

A 60 ml plastic syringe results in a core sample with a standard area of 5.6 cm^2. *Confirm area of syringe prior to use*. If compositing is used, the number of cores composited must be confirmed from the SVF/COC. Calculate as follows:

$$\frac{5.6\ cm^2 \times "number of cores"}{10{,}000\ cm^2 / m^2} = X\ m^2$$

For example, for one core: $\frac{5.6\ cm^2 \times 1}{10{,}000\ cm^2 / m^2} = 0.00056\ m^2$

6.2.3. Area of Templates

Templates may vary from the 12.5 cm^2 size standard. Confirm area of template prior to use. All template samples must list the size of the area scraped on the SVF/COC.

$$\frac{12.5\ cm^2 \times "number of templates"}{10{,}000\ cm^2 / m^2} = X\ m^2$$

For example, for one template: $\frac{12.5\ cm^2 \times 1}{10{,}000\ cm^2 / m^2} = 0.00125\ m^2$

6.2.4. Reach Wide Chlorophyll-a Composite Calculation

Each type of sample will contribute more or less to the sample as a whole depending upon the number of like-kind samples composited. A weighted average of chlorophyll-a is determined from the following equation.

$\sum$ **[(Rc*Nc)+(Rt*Nt)+(Rh*Nh)]/[(Nc+Nt+Nh)]**

Where: R(c,t,h) = Chlorophyll-a mean result from a core, template, and or hoop
N(c,t,h) = Number of each type

For example. Assume: 3 cores were taken (Average Chloropyll-a = 20.0 mg/m^2
6 templates (Average Chloropyll-a = 60.0 mg/m^2)
2 hoops (Average Chloropyll-a = 80.0 mg/m^2)

	# of Type	R
Cores:	3	20.0
Templates:	6	60.0
Hoops:	2	80.0

Therefore: $\sum$ **[(20*3)+(60*6)+(80*2)]/11**

Reach Weighted Average for Chloropyll-a = 52.7 mg/m^2

7. Determination of Chlorophyll-a by HPLC

With DEQ QA officer approval, Standard Methods10200 H(5) or EPA Method 447.0 may be used if the laboratory confirms the data generated by HPLC compares to the spectrophotometric method. All method specific QA/QC protocols must be followed. For calculation under SM 10200 H(5) Section 9 use the data from Table 1 of this document.

8. Reporting Results

Results of Chlorophyll-a analyses must be reported in conformance with Bureau SOP WQPBDMS-010.

Literature Cited

APHA. 1998. *Standard Methods for the Examination of Water and Wastewater*.: American Public Health Association.

Arar E. 1997. EPA Method 446.0 - *In Vitro* Determination of Chlorophylls a, b, $c_1 + c_2$ and Pheopigments in Marine And Freshwater Algae by Visible Spectrophotometry, Revision 1.2. Washington, D.C.: U.S. Environmental Protection Agency.

Lazorchak JM, Klemm DJ, and Peck DV. 1998. Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods For Measuring the Ecological Condition Of Wadeable Streams. Washington D.C.: U.S. Environmental Protection Agency.

Sartory D.P., and Grobbelaar J.U. 1984. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis., 177-187. The Hague: Dr. W. Junk Publishers.

Suplee MW. 2004. Wadeable Streams of Montana's Hi-line Region: An Analysis of Their Nature and Condition, with an Emphasis on Factors Affecting Aquatic Plant Communities and Recommendations to Prevent Nuisance Algae Conditions. Helena, MT: Montana Department of Environmental Quality.

Suplee MW, Watson V, and et al. 2006. An Experiment to Determine the Efficiency of Measuring Chlorophyll a in Stream Sediment Samples using Spectrophotometric Methods. Montana Department of Environmental Quality.

Attachment 1

Site Visit Form/Chain-of-Custody

Place Site Visit Label Here

Site Visit Form

(One Station per page)

STORET Project ID:______________

STORET Trip ID :______________

Date: ______________ **Time:** ______________ **Personnel:** ______________

Waterbody:______________ Location: ______________

Station ID: ______________ Visit #: ________ HUC: ______________ County: ______________

Latitude: ___ ___ . ___ ___ ___ ___ Longitude: ___ ___ ___ . ___ ___ ___ ___ Lat/Long Verified? ☐ By:

Elevation (m): ______________ Geo Method: **GPS** Other:__________ Datum: NAD27 **NAD83** WGS84

Samples Collected:		**Sample ID** (Provide for **all** samples)**:**	**Sample Collection Information/Preservation:**
Water	☐		GRAB EWI
Analysis:			Preserved: HNO_3 H_2SO_4 H_3PO_4 HCL None
Analysis:			Preserved: HNO_3 H_2SO_4 H_3PO_4 HCL None
Analysis:			Preserved: HNO_3 H_2SO_4 H_3PO_4 HCL None
Analysis:			Preserved: HNO_3 H_2SO_4 H_3PO_4 HCL None
Analysis:			Preserved: HNO_3 H_2SO_4 H_3PO_4 HCL None
Analysis:			Preserved: HNO_3 H_2SO_4 H_3PO_4 HCL None
Analysis:			Preserved: HNO_3 H_2SO_4 H_3PO_4 HCL None
Sediment	☐		SED-1
Analysis:			Preserved: None Other:
Analysis:			Preserved: None Other:
Chlorophyll a	☐		Method: C=Core H=Hoop T=Template N=None Location: R=Right C=Center L=Left
Composite at Lab ☐			
Transect: A___-___ B___-___ C___-___ D___-___ E___-___ F___-___ G___-___ H___-___ I___-___ J___-___ K___-___			
Phytoplankton	☐		D1 Filtered:______mL D2 Filtered:______mL
Algae	☐		PERI-R PERI-1 OTHER:
Macroinvert.	☐		KICK HESS JAB OTHER:
Kick/Jab Length (ft):	Kick Duration/# Jabs:	# of Jars:	Mesh Size: 1200 1000 500 OTHER:

Field Measurements:		**Field Assessments:**
Water Temp: °C °F	Air Temp: °C °F	Macroinvertebrate Assessment ☐
pH:	SC: (umho/cm)	Habitat Assessment: Reach ☐ Site ☐ EMAP ☐
DO: (mg/L)	Flow: (cfs)	Substrate: Pebble Count ☐ Percent Fines ☐ RSI ☐
Flow Comments: Dry Bed ☐ No Measurable Flow ☐		Channel Cross-Section ☐
Flow Method: Meter ☐ Float ☐ Gage ☐ Visual Est. ☐		Photographs: Digital ☐ Film ☐
Turbidity: Clear ☐ Slight ☐ Turbid ☐ Opaque ☐		Data Logger: Temperature ☐ YSI ☐ Weather Station ☐

Site Visit Comments:	

Chemistry Lab Information:		
Lab Samples Submitted to:	Account #:	Date Submitted:
Invoice Contact & Address:		
Contact Name & Phone:		
EDD ☒ Format: SIM Compatible	Term Contract Number:	
Relinquished By & Date/Time:	Shipped By & Date/Time:	Received By & Date/Time:
Relinquished By & Date/Time:	Shipped By & Date/Time:	Received By & Date/Time:

Lab Use Only - Delivery Temperature: Wet Ice______°C Dry Ice______°C

Site Visit Form Instructions

1. Place a Site Visit Code label in the upper left corner (ONLY 1 SITE VISIT CODE PER FORM).
2. Place a Trip Label in the upper right corner. (Covering Project ID and Trip ID with label is alright.)
3. **STORET Project ID**: If you do not have a Trip Label, enter the Project ID assigned by Data Management. If Project ID is not assigned, leave blank for STORET Database Manager.
4. **Trip ID:** If you do not have a Trip Label, enter the Trip ID assigned by Data Management. If Trip ID is not assigned, leave blank for STORET Database Manager.
5. **Date/Time**: Enter the date and time of the station visit.
6. **Personnel**: Enter the first and last name(s) of the personnel conducting field activities.
7. **Waterbody**: Enter the name of the waterbody such as "Missouri River".
8. **Location**: Description of sample location such as "upstream from bridge on Forest Service road 100". For confidentiality please DO NOT use proper names of people in the location field.
9. **Station ID**: If you have a Trip Label, enter the established ID. If there is no ID on the Trip Label, leave the field blank and Data Management will generate a Station ID when the SVF is submitted.
10. **Visit #**: Enter "1" if this is a new station. Leave blank if visit number is unknown.
11. **HUC**: If you do not have a Trip Label, enter the fourth code (8 digit) HUC the station falls within.
12. **County**: If you do not have a Trip Label, enter the county in which the station falls within.
13. **Lat/Long**: Latitude and Longitudes should be obtained in decimal degrees using a GPS unit reading **NAD83** whenever possible. If a lat/long is obtained by another method, the datum and method must be recorded in the Site Visit Comments.
14. **Lat/Long Verified**: Latitudes and Longitudes should be verified immediately upon return from the field. Verify by plotting on a paper map or using a mapping website. Once the lat/long has been verified check the Verified box and enter initials after "By".
    - Do not make minor adjustments to measured values during verification; they are assumed to be correct within the limitations of the measurement system.
    - Gross errors should be corrected as follows: 1) Draw a single line through the erroneous value(s) and initial. Do not erase the original reading. 2) Write the corrected value in the comment field along with the method and datum used to derive the corrected value.
15. **Elevation:** Record elevation collected by GPS and circle the GPS datum used. If elevation is obtained by another method, the datum and method must be recorded in the Site Visit Comments.
16. **Samples Collected**: Check the box next to each activity that is collected during the station visit.
17. **Sample ID**: Write the Sample ID (Site Visit Code-sample identifier) for all of the samples collected.
18. **Sample Collection Procedure**: Circle the appropriate Sample Collection Procedure ID.
    - For each Chlorophyll a transect, record the sample collection method in the first space provided and the sample location in the second space provided (example: A: T - R).
    - For Phytoplankton, record the volume filtered for each sample collected.
19. **Analysis Requested**: Record the requested laboratory analysis for each chemistry sample and circle the preservative used.
20. **Field Measurements**: Record your field measurements in the spaces provided.
21. **Field Assessments**: Check the boxes next to each type of field assessment completed.
22. **Site Visit Comments**: Record general comments about the station visit, samples, and field measurements.
23. **Chemistry Lab Information**: If chemistry lab samples were taken, complete this section.
    - Lab Samples Submitted to: Enter name of laboratory where samples will be sent.
    - Account #: Enter account number at laboratory where samples will be sent.
    - Date Submitted: Record date the samples were received by the laboratory.
    - Sign and date the form each time the samples change possession.

ATTACHMENT 2

SOP WQPBWQM-011
EMAP REACH FORM

EMAP REACH FORM

WATERBODY NAME:_____ **LOCATION:**_____

SITE ID: _____ **PERSONNEL:** _____ **DATE:** _____

STREAM/RIVER REACH DETERMINATION			
Channel Width Used to Define Reach (m)	DISTANCE (m) FROM F-SITE		Comment
	Upstream Length	Downstream Length	

SKETCH MAP – Arrow Indicates North